IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Jonathan M. Graff et al.
Serial No.	:	10/002,631
Filing Date	:	October 31, 2001
Title	:	METHOD TO IDENTIFY SIGNAL SEQUENCES
Group Art Unit	:	To be assigned
Examiner	:	To be assigned.

PETITION TO MAKE SPECIAL UNDER 37 C.F.R. § 1.102(d)
ACCELERATED EXAMINATION

April 9, 2002

BY EXPRESS MAIL NO. EU206443033US

BOX PATENT APPLICATION

Assistant Commissioner
for Patents
Washington, D.C. 20231

Applicants petition hereby to make the above-referenced new patent application special in order to accelerate the examination of the application. Applicants concurrently submit herewith an executed Oath and Declaration by the inventors, a Preliminary Amendment, a Petition to Correct Inventorship and the required fees.

A. Applicant's Claims Are Directed To A Single Invention

Applicant believes that all claims are directed to a single invention. Further, Applicant will make an election without traverse as a prerequisite to the grant of special status should the U.S. Patent and Trademark Office determine that all of the claims presented are not obviously directed to a single invention.

B. Applicant Has Caused A Pre-Examination Search To Be Made

Applicant has caused to be conducted a pre-examination search. The search was conducted in the following classes/subclasses:

Class/subclass
435
435/5
435/6
435/34
435/68
435/69.1
435/91
536

The following relevant prior art listed by United States Patent Number (class/subclass), Foreign Patent Number (class/subclass) or by publication information was uncovered by the search, copies of which are enclosed:

U.S. PATENTS

Patent	Class/subclass	Inventor(s)	Title
6,136,569	435/91.41	Baker et al.	METHOD OF CREATING A cDNA LIBRARY ENRICHED IN SIGNAL SEQUENCES
6,103,472	435/5	Thukral	METHODS AND COMPOSITIONS FOR IDENTIFYING NOVEL SECRETED MAMMALIAN POLYPEPTIDES IN YEAST

6,060,249	435/6	Baker et al.	METHOD OF SELECTING FOR GENES ENCODING SECRETED AND TRANSMEMBRANE PROTEINS
5,952,171	435/69.8	McCarthy et al.	METHOD FOR IDENTIFYING GENES ENCODING SECRETED OR MEMBRANE-ASSOCIATED PROTEINS
5,712,116	435/69.1	Jacobs	METHOD FOR ISOLATING CYTOKINES AND OTHER SECRETED PROTEINS
5,536,637	435/6	Jacobs	METHOD OF SCREENING cDNA ENCODING NOVEL SECRETED MAMMALIAN PROTEINS IN YEAST
5,212,070	435/6	Smith et al.	SECRETORY SIGNAL SELECTION VECTORS FOR EXTRACELLULAR PROTEIN SYNTHESI IN BACILLI

FOREIGN PATENTS

Patent	Country	Inventor(s)	Title
WO 97/40146	PCT	JACOBS	YEAST INVERTASE GENE AS REPORTER SYSTEM OF ISOLATING CYTOKINES
244,042	Europe	Smith et al.	SECRETORY SIGNAL SELECTION VECTORS FOR EXTRACELLULAR PROTEIN SYNTHESIS IN BACILLI

OTHER PUBLICATIONS

Ravn et al. (2000) The development of TnNuc and its use for the isolation of novel secretion signals in Lactococcus lactis. *Gene* 242(1-2):347-356.

Uratani et al. (1995) Construction of secretion vectors and use of heterologous signal sequences for protein secretion in Clavibacter cyli subspecies cynodontis. *Mol. Plant Microbe Interact* 8(6):892-898.

Hols et al. (1994) Use of homologous expression-secretion signals and vector-free stable chromosomal integration in engineering of Lactobacillus plantarum for alpha-amylase and levanase expression. *Applied and Environmental Microbiology* 60(5):11401-1413.

Perez-Martinez et al. (1992) Protein export elements from Lactococcus lactis. *Mol. Gen. Genet.* 234(3):401-411.

Bielefeld and Hollenberg (1992) Bacterial beta-lactamase is efficiently secreted in Sacchromyces cerevisiae under control of the invertase signal sequence. *Current Genetics* 21(4/5):265-268.

Sibakov et al. (1991) Secretion of TEM beta-lactamase with signal sequences isolated from the chromosome of Lactococcus lactis subspecies lactis. *Applied and Environmental Microbiology* 57(2):341-348.

Smith et al. (1988) Characterization of signal-sequence-coding regions selected from the Bacillus subtilis chromosome. *Gene* 70(2):351-361.

These references are discussed and distinguished below.

C. Detailed Discussion of References

1. United States Patents

United States Patent No. 6,136,569, issued to Baker et al. on October 24, 2000 ("the '569 patent") describes, in relevant part, a method for identifying cDNA's which encode secreted and membrane-bound proteins in yeast. The method comprises providing to a yeast cell which cannot degrade starch, a DNA containing a coding sequence of a mammalian signal peptide ligated to a DNA encoding an amylase lacking a functional native signal peptide and determining whether the yeast cell has the ability to degrade starch, wherein the ability to degrade starch is dependent upon the presence of functional amylase and wherein the function of the amylase is dependent upon the presence of a functional signal sequence. This method allows for the identification of mammalian signal sequences in a yeast system.

Similarly, United States Patent No. 6,103,472, issued to Thukral on August 15, 2000 ("the '472 patent") describes, in relevant part, a method for identifying novel secreted mammalian proteins in yeast. The method comprises providing to a yeast cell which cannot utilize starch, a vector containing (a) a DNA encoding a mammalian signal peptide and (b) a DNA encoding an α-amylase lacking a signal sequence and selecting for yeast cells which can utilize starch in growth medium, wherein the ability to utilize starch is dependent upon the

presence of functional amylase and wherein the function of the amylase is dependent upon the presence of a signal sequence. The '472 patent states that "signal sequence trapping using mammalian cells has disadvantages, including low transfection efficiency and difficult recovery of a desired clone from cells that have been transfected with multiple plasmids. **Procaryotic cells have a secretion pathway which is not suitable for identifying mammalian secretion signals**." *Emphasis added. See* column 2, lines 12-17. The '472 patent also describes a method identifying mammalian signal sequences in a yeast system.

United States Patent No. 6,060,249, issued to Baker et al. on May 9, 2002 ("the '249 patent"), also describes a method for identifying DNAs which encode secreted and membrane bound proteins comprising providing to a yeast cell, which cannot degrade starch, a DNA encoding a mammalian signal sequence and encoding an amylase gene lacking a signal sequence and identifying yeast cells which have a functional amylase by their ability to degrade starch, wherein the function of the amylase is dependent upon the presence of a signal sequence.

United States Patent No. 5,952,171, issued to McCarthy et al. on September 14, 1999 ("the '171 patent"), describes a method for identifying a cDNA encoding a mammalian protein having a signal sequence comprising providing to bacterial cells a library of mammalian cDNA ligated to a DNA encoding an alkaline phosphatase lacking a signal sequence, isolating the DNAs from the bacteria, transfecting mammalian cells which do not express alkaline phosphatase with the DNAs, screening the mammalian cells for the presence of phosphatase activity. This method allows for identifying mammalian signal sequences using a cumbersome two-step system which employs both bacterial cells and mammalian cells.

United States Patent No. 5,712,116 and 5,536,637, issued to Jacobs on January 27, 1998 and July 16, 1996, respectively ("the '116 patent" and "the '637 patent" respectively) describe a method for isolating mammalian proteins (cytokines and other secreted proteins) using a yeast based system which employs a mammalian cDNA library ligated to a DNA encoding a yeast invertase that is not secreted that is prepared in *E. coli* and then used to transform yeast cells. The yeast cells that comprise a mammalian DNA signal sequence are selected by growth on sucrose or raffinose because the cells will only grow on such medium if the invertase is

secreted. The method requires the use of yeast cells lacking SUC2. In addition, as indicated in the '249 and '569 patents listed above, false positives are possible because the yeast cells may grow on sucrose and raffinose in the absence of secreted invertase, although at a slower rate. This method also identifies mammalian signal sequences using a yeast system.

United States Patent No. 5,212,070, issued to Smith et al. on May 18, 1993 ("the '070 patent") describes vectors which are useful for identifying secretory signal sequences from DNA fragments of prokaryotic unicellular microorganisms. The identified prokaryotic signal sequences are used to produce proteins in secreted form by ligating a DNA encoding the signal sequences to a DNA encoding another product for the purpose of expressing and secreting large amounts of heterologous proteins. The vectors were constructed such that the presence of a prokaryotic (*Bacilli*) signal sequence results in a secreted β-lactamase (or α-amylase or penP) which is detectable by the ability of the bacterial cells to grow on plates containing ampicillin. The prokaryotic signal sequences so identified are then used for producing secreted forms of heterologous proteins in *Bacilli*. The 070 patent describes a method for identifying prokaryotic signal sequences in a prokaryotic system.

2. Foreign Patents and Patent Publications

PCT Publication No. WO 97/40146, published on October 20, 1997 of the Genetics Institute, Inc. is the corresponding PCT application to United States Patent No. 5,712,116 and describes the method indicated above.

European Patent Application No. 244,042, published November 4, 1987, of Gist-Brocades N.V. corresponds to United States Patent No. 5,212,070 described above.

3. Other Publications

Ravn et al., in *Gene* 242:347-356 (2000), describe a method for identifying secretion signals from *Lactococcus lactis*, a gram positive bacteria. The method employs the development of a new Tn917-transposon derivative, termed TnNuc, which includes the *Staphylococcus aureus* nuclease gene (nuc) as a reporter for secretion. Transposition of TnNuc into the *Lactococcus lactis* chromosome allows for the generation of fusions in-frame with the

nuc gene. A reporter gene (lacZ) is also present. *Lactococcus lactis* having Lac+ phenotype result from transposition of TnNuc into a functional gene on the *Lactococcus lactis* chromosome. The presence of a functional signal sequence upstream from TnNuc allows for the detection of nuclease activity using a plate assay. This method is specific for *Lactococcus lactis* because it requires transposition of TnNuc into the *Lactococcus lactis* chromosome for detecting *Lactococcus lactis* signal sequences. The Ravn et al. method allow for identification of prokaryotic signal sequences in a prokaryotic system.

Uratani et al. in *Mol. Plant Microbe Interact.* 8:892-898 (1995), describe the construction of vectors useful for determining whether heterologous signal sequences are functional in *Clavibacter xyli subsp. cynodontis*. The vector includes the PhoA gene from *E. coli* from which promoter and signal sequences have been deleted. Known signal sequences from *Streptomyces* origin were cloned into the vector and the level of secreted alkaline phosphatase was determined. The presence of secreted alkaline phosphatase requires that a functional signal sequence be present in PhoA. Uratani et al. showed that a number of known *Streptomyces* signal sequences were functional in *Clavibacter xyli subsp. cynodontis* and hypothesize that the system is useful for detecting endophytic bacteria in some locations in planta and may be useful for the study of plant-microbe interactions. Uratani et al. do not describe a method for identifying signal sequences, but rather describe a method for determining whether a known bacterial reporter gene from a first bacterial species can be combined with signal sequences from a second bacterial species and can be successfully expressed in a third bacterial species.

Hols et al. in *Applied and Environmental Microbiology* 60:1401-1413 (1994), describe the isolation and characterization of expression and secretion signals from *Lactobacillus plantarum*. Hols et al. constructed a vector containing an amylase gene deleted of its own signals governing expression (promoter) and secretion (signal peptide). *Lactobacillus plantarum* transfected with this vector were amylase negative. Expression could be restored by cloning DNAs encoding expression and signal sequences in front of the amylase gene. A library of *Lactobacillus plantarum* cDNA was cloned into the vector containing the truncated amylase gene and this vector was used to transform *E. coli*. *E. coli* that could grow on plates including starch were selected as positive clones since the ability of growing on starch is dependent upon

the presence of a functional amylase gene. The isolated clones were sequenced to identify expression and signal sequences. Hols et al. identified the expression and signal sequences in order to develop better *Lactobacillus plantarum* expression systems. It was previously known that the wild-type amylase gene did not function well in *Lactobacillus plantarum* and therefore was not useful as a reporter gene. Hols et al. show that this is due to the inability of *Lactobacillus plantarum* to recognize the native expression and signal sequences and further showed that by replacing these sequences with *Lactobacillus plantarum* sequences, amylase is made and secreted and the *Lactobacillus plantarum* can then grow on plates including starch. This method allows for the identification of prokaryotic signal sequences in a prokaryotic system.

Perez-Martinez et al. in *Mol. Gen. Genet.* 234:401-411 (1992), describe signal sequences from *Lactococcus lactis*. Perez et al. used vectors containing α-amylase and β-lactamase reporter genes lacking signal sequences to identify signal sequences from *Lactococcus lactis*. DNAs containing sequences encoding signal sequences were selected in *E. coli* by their ability to restore α-amylase and β-lactamase activity therein. This is another demonstration of a method for identifying prokaryotic signal sequences in a prokaryotic system.

Bielefeld and Hollenberg, in *Curr. Genet.* 21:265-268 (1992) show that fusion of bacterial β-lactamase to the signal sequence of yeast invertase leads to the efficient secretion of β-lactamase in yeast. Generally, the bacterial β-lactamase is not efficiently secreted in yeast. Bielefeld and Hollenberg showed that the bacterial signal sequence did not efficiently secrete the β-lactamase in yeast, presumably due to the incompatibility of the bacterial sequences in yeast. Bielefeld and Hollenberg do not describe a method for identifying signal sequences but rather show that yeast signal sequences can be used to replace bacterial signal sequences of bacterial proteins and the resultant fusion can be efficiently secreted in yeast.

Sibakov et al. in *Applied and Environmental Microbiology* 57:341-348 (1991) describe the identification of expression and signal sequences from *Lactococcus lactis* in *E. coli*. Sibakov cloned fragments of the *Lactococcus lactis* genome into a DNA containing a β-lactamase gene lacking expression and signal sequences and transformed the DNA into *E. coli*

and screened for the ability of the *E. coli* to grow on ampicillin containing plates. The presence of a *Lactococcus lactis* signal sequence restored the activity of the β-lactamase and conferred ampicillin resistance. Several signal sequences were isolated. The method described by Sibakov et al. also allows for the identification of prokaryotic signal sequences in a prokaryotic system.

Smith et al. in *Gene* 70:351-361 (1988) also describe the selection of bacterial signal sequences of *Bacillus subtilis* in a bacterial system (*E. coli*). As did Sibakov et al., Smith et al. employed a β-lactamase gene lacking expression and signal sequences to identify *Bacillus subtilis* signal sequences.

D. Distinction between Applicant's claimed invention and the Above-Described References

None of the references discussed above disclose or suggest a method for identifying eukaryotic signal sequences, and particularly those from mammals, in a prokaryotic system as described in the present invention.

Methods are known for identifying exogenous eukaryotic signal sequences in yeast, a eukaryote, but such methods are cumbersome and are prone to false positives. In addition, methods are known for identifying prokaryotic signal sequences in prokaryotic systems. It is believed however that such prokaryotic systems are not useful for the identification of eukaryotic signal sequences because "**procaryotic cells have a secretion pathway which is not suitable for identifying mammalian secretion signals**." *Emphasis added. See* United States Patent 6,103,474, column 2, lines 12-17.

The present invention provides for a method for identifying and/or obtaining eukaryotic signal sequences using a prokaryotic system. Prokaryotic systems are more efficient and less error prone than their yeast counter-parts and therefore, it is desirable to utilize such methods for the identification of eukaryotic signal sequences. The present invention provides a method for identifying and/or obtaining a candidate eukaryotic nucleic acid encoding a polypeptide which comprises a signal sequence and/or transmembrane sequence comprising:

(a) contacting a bacterial cell with a plasmid comprising a marker gene and a candidate eukaryotic nucleic acid; and

(b) screening for function of the marker gene, wherein the function of the marker gene requires the presence of a signal sequence and/or a transmembrane sequence. The method may further comprise isolating and characterizing the candidate eukaryotic signal sequence. The method has been used to identify, isolate and characterize numerous eukaryotic signal sequences. Such signal sequences are useful for the diagnosis of diseases and for the development of new treatments for diseases. While similar systems have been described which utilize yeast-based systems for the identification of mammalian signal sequences and bacterial-based systems for the identification of bacterial signal sequences, no one has described a method for the identification, isolation and characterization of eukaryotic signal sequences using a bacterial system as described by the above-referenced patent application. Furthermore, as noted above, it is actually believed that such prokaryotic systems are not useful for the identification of eukaryotic signal sequences due to the differences in the signal pathways.

Conclusion

By this Request for Expedited Examination, Applicants respectfully request that examination of the above-identified application be expedited pursuant to 37 C.F.R. §1.102(d). Enclosed is a check in the amount of $130.00 to cover the petition fee pursuant to 37 C.F.R. §1.102(d) . The Commissioner is hereby authorized to charge any additional fees or credit any overpayment to Deposit Account No. 02-4377 of Baker Botts L.L.P.

Early and favorable acceptance of this petition and application is respectfully requested.

Respectfully submitted,

April 9, 2002
Dated

Rochelle K. Seide
Reg. No. 32,300

Alicia A. Russo
Reg. No. 46,192

Attorneys for Applicants

BAKER BOTTS L.L.P.
30 Rockefeller Plaza, 44th Floor
New York, New York 10112
(212) 408-2500